

Towards measles elimination in Italy: Virological surveillance and genotypes trend (2013–2015)

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ABSTRACT

In accordance with the goal of the World Health Organization Regional Office for Europe, the Italian National Measles and Rubella Elimination Plan aimed to interrupt indigenous measles transmission in Italy by the end of 2015. However, from 2013 to 2015, Italy experienced high measles burden with 4902 measles cases (49.3% laboratory-confirmed) reported to the enhanced measles surveillance system (cumulative incidence in the triennium reference period: 2.4/100,000 population). The measles elimination goal was not reached.

Laboratory surveillance of measles circulating genotypes is performed by the Measles and Rubella National Reference Laboratory (NRL) at the Italian National Institute of Health (Istituto Superiore di Sanità – ISS), in Rome. Samples received from 1 January 2013–31 December 2015 were analysed. Those positive for measles genome by molecular tests were sequenced and phylogenetically analysed. Phylogenetic analysis performed by NRL identified that genotypes D4 and D8 were endemic and co-circulated in 2011–2013: study results show that genotype D4 disappeared during 2013. Sporadic cases were associated to genotype B3 during 2011–2013, which became endemic in Italy during 2014 and co-circulated with D8 until 2015. Sporadic cases were found belonging to genotypes D9 and H1 all over the period in exam. Similar trend has been observed in European WHO Region.

1. Introduction

Measles virus (MV), member of the Paramyxoviridae family, is a leading cause of mortality among young children worldwide, with an estimated 134,200 deaths due to measles-related complications in 2015 (WHO, 2017a). Although measles is a highly contagious viral disease, its transmission can be easily prevented by vaccination.

In order to reach measles elimination, a very high coverage of at least 95% for two doses of vaccine is needed (WHO, 2012a). A monovalent measles vaccine was introduced in Italy in 1976. This was replaced in 1993 by the combined measles-mumps-rubella (MMR) vaccine, given as a single dose at 13–15 months of age, but only since 1999, the vaccination with MMR has been included in the national immunization program. Currently, a first dose of MMR vaccine is

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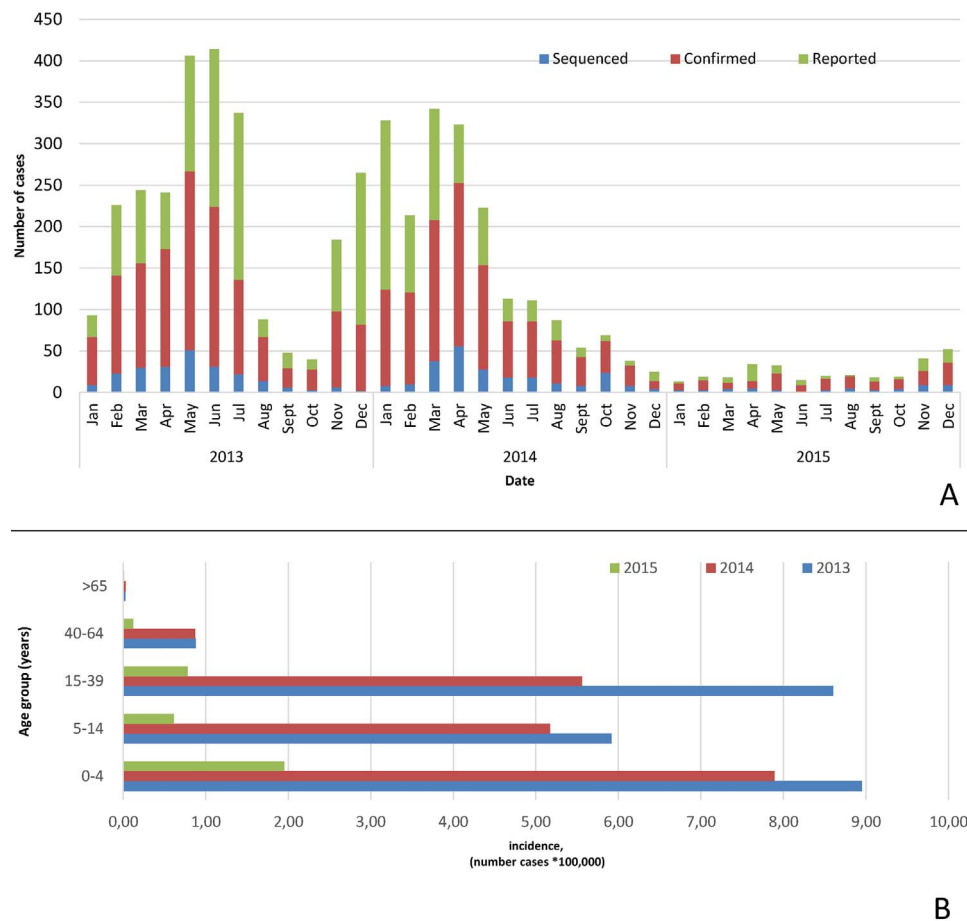


Fig. 1. Measles cases by month of rash onset (A) and incidence by age group (B), Italy, 2013–2015.

recommended at the age of 12–15 months and a second dose at 5–6 years (Filia et al., 2013). National administrative vaccine coverage for the first dose of MMR vaccine in children at two years of age, decreased from 90.4% in 2013–86.7% in 2014 and 85.3 in 2015 due to the economic crisis and a completely discredited health scare linking the MMR jab to autism.

The World Health Organization (WHO) European Regional Committee set 2015 as target date for the elimination of measles (WHO, 2010): regional elimination can be declared after 36 or more months of the absence of endemic measles or rubella in all Member States (Mankertz et al., 2011).

Currently, eliminating measles and rubella is a core goal of the European Vaccine Action Plan 2015–2020.

Based on a country-by-country assessment, during its fifth meeting in 2016, the European Regional Verification Commission for Measles and Rubella Elimination (RVC) concluded that based on reports submitted, at the end of 2015, endemic measles transmission had been interrupted in 37 of the 53 Member States (70%). Twenty-four Member States (45%) provided evidence to demonstrate the elimination of endemic transmission of measles for at least 36 months; further 13 Member States (25%) provided evidence for the interruption of measles transmission for a period of less than 36 months. Fourteen Member States (26%) were considered by the RVC to remain endemic for measles transmission, including Italy (WHO, 2017b).

Fourteen Member States (26%) were considered by the RVC to remain endemic for measles transmission, and 16 (30%) were considered to remain endemic for rubella transmission. Fourteen Member States (26%) were considered to remain endemic for both measles and rubella. WHO's plan to eliminate measles seeks to improve measles surveillance systems throughout the world by WHO Measles and

Rubella Laboratory Network (Mulders et al., 2016), in order to detect all clinical measles cases and to investigate thoroughly every single cases and outbreaks. In Italy, the surveillance of measles consists of a case reporting system based on epidemiological investigation on cases clinically consistent with measles supported by laboratory confirmation. Analysis of samples for laboratory confirmation of infection is performed by the National Reference Laboratory (NRL) at the National Institute of Health and by Subnational Reference Laboratories (SRLs) not WHO accredited. The reporting and laboratory surveillance system has been previously described (Magurano et al., 2015).

Virus surveillance and genetic characterization of circulating viruses are important tools for regional and global control efforts. Within the WHO's goal to eliminate the virus, the NRL plays a key role in supporting cases ascertainment in Italy, confirming outbreaks/cases and determining MV circulating genotypes.

This report describes the Italian virological surveillance for measles in the triennium 2013–2015.

2. Material and methods

2.1. Epidemiological data

Since 1934, measles disease has been statutorily notifiable in Italy. In 2007, an enhanced surveillance system was introduced which requires physicians to report all suspected measles cases to the local health authorities within 12 h (Italian Ministry of Health, 2007).

For each suspected case, the local health authorities are required to carry out an epidemiological investigation, to obtain specimens for laboratory confirmation and genotyping, and complete a standard measles notification process on the online database of the National

2.2. Laboratory data

Urine, oral fluid (OF) and dried blood samples were collected after onset of rash from suspected measles cases according to WHO recommendations (WHO, 2007). Serological and molecular diagnosis were performed on these samples as previously described (Magurano et al., 2015). Sequences were obtained amplifying by RT-PCR a fragment of the carboxyl-terminal coding region of the N gene, including the 450 bp recommended for genotyping. Sequences provided by SRLs were also included in this study.

Sequence data were analyzed by BioEdit (version 7.0). Bayesian information criterion (BIC) was used to determine the model of nucleotide substitution that best fit the data using the selection tool available in MEGA6 (Tamura et al., 2013). The model that best fit the data was the Kimura 2-parameter (k2) model. Phylogenetic analyses were performed using MEGA version 6 by the Kimura 2 (K2) method by 1000 resampling. MV strains were named as designated by WHO (WHO, 2012b). The sequences reported in this article have been deposited into the WHO's MeaNS database (Rota et al., 2011).

3. Results

From January 2013 to December 2015, 4902 cases were reported in Italy. Of these 2416 were laboratory-confirmed (Fig. 1A) by serological and/or molecular methods performed on dried blood spots and urine/OF samples, respectively.

The cumulative incidence in the three-year period was 2.4 cases per 100,000 population, the data were supported by statistical analysis (4.1, 2.7 and 0.4 cases per 100,000 in 2013, 2014 and 2015, respectively).

Fig. 1B shows the age group distribution and incidence of measles cases in Italy for the period 2013–2015. The highest incidence was found in the age group 0–4 years followed by the age group 15–39 years, these groups present the higher number of measles-susceptibles. Median age of cases was 22 years (range: 0–83 years).

Overall, samples were collected from 2848 suspected measles cases and for 359 of these the vaccination was available, 245 and 79 were vaccinated with a single and two doses, respectively. All the samples were tested either by serological or molecular methods. PCR-positive samples coming from the regions where measles cases were reported were sequenced. A total of 484 sequences from different Italian regions were phylogenetically analysed by the NRL: 167 in 2013, 260 in 2014 and 57 in 2015. Fig. 2A shows the genotypes distribution by year.

Sequence analysis identified the co-circulation of genotype D4 and D8 in the year 2013 similarly to 2011 and 2012 (data not shown). No cases were found to belong to genotype D4 in 2014, when the genotype D8 circulated together with genotype B3. Sporadic cases belonged to genotypes D9 and H1 during the years 2013 and 2014. The Italian trend of MV genotypes distribution by each year is shown in Fig. 2A.

3.1. D4

NRL's results showed that D4 was endemic in 2010 (Baggiere et al., 2014) and continued to be endemic until the first half of 2013. The phylogenetic tree (Fig. 3A) shows that all D4 MV strains of 2013 grouped into 2 different clusters. Each cluster shows a 100% identity with a "WHO single named variant": the Manchester (MVs/Manchester.GBR/10.09/) and the Iasi (MVs/Iasi.ROU/12.12/) variants.

Genotype D4 was not observed in 2014, and only 3 sporadic cases were identified in 2015. Epidemiological data revealed that two of these cases (identified in the province of Parma) were imported from India.

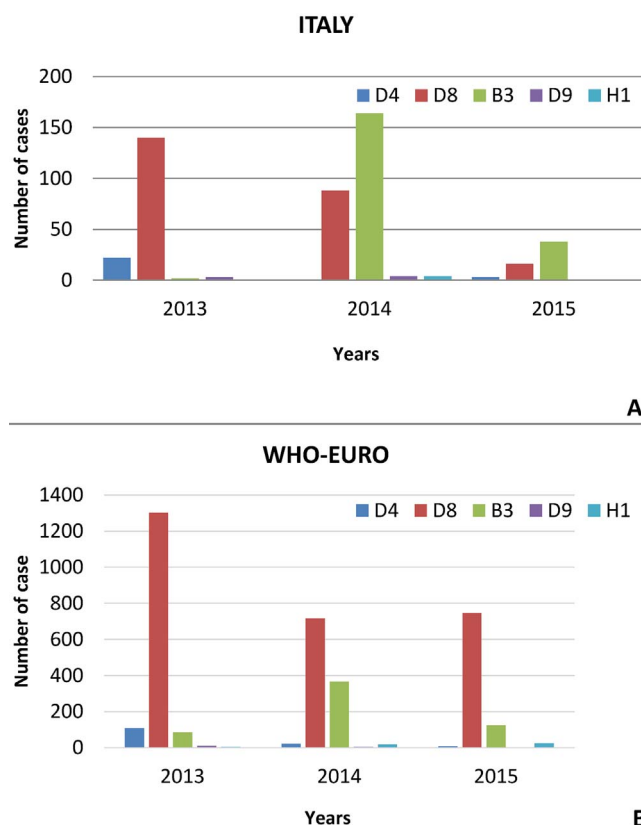


Fig. 2. Italian (A) and European (B) measles genotypes trend from 2013 to 2015.

3.2. D8

Beside genotype D4, D8 circulated in Italy during 2011 and 2012 (Magurano et al., 2015) and increased in 2013 with a rate of presence of 83.8% (140/167). During 2014, D8 circulated with genotype B3, with 88 cases leading the percentage to 33.8% (88/260). In 2015 genotype D8 was responsible of 16 out of 57 (28%) cases characterized.

The phylogenetic tree (Fig. 4A) shows that all D8MV strains in 2013 grouped into 3 different cluster. Each cluster shows a 100% identity with a WHO named variant: the Taunton (MVs/Taunton.GBR/27.12/), the Frankfurt-Main (MVs/Frankfurt-Main.DEU/17.11/) and the Villupuram (MVi/Villupuram.IND/03.07/) variants.

Five clusters with five related WHO named variant were identified in 2014 (Fig. 4B): the Taunton, the Frankfurt Main, Hulu Langat (MVi/HuluLangat.MYS/26.11/), Rostov on Don (MVs/Rostov on Don.RUS/47.13/2) and Villupuram. One unnamed variant was also identified. Data of 2014 on the circulation of genotype D8 in Northern Italy (Lombardy) have been reported (Amendola et al., 2017).

In 2015 two main clusters of D8 were identified: one of these showed a 100% identity with the WHO variant "Rostov on Don" (MVs/Rostov on Don.RUS/47.13/2), and the second showed an identity of 100% with the "Villupuram" WHO variant.

In summary, "Taunton" was the most representative variant during 2013 – 2014, as it has been identified from 39 and 28 cases, respectively. Strains belonging to this variant circulated in the Lombardy, Piedmont, Friuli-Venezia Giulia, Veneto, Trentino-Alto Adige, Marche, Emilia-Romagna, Tuscany, Umbria, Abruzzo, Campania and Apulia Italian regions in 2013 and in Lombardy, Piedmont, and Calabria in 2014. In the same years, the "Frankfurt-Main" variant was identified in Lombardy, Veneto, Friuli-Venezia Giulia, Trentino-Alto Adige, Piedmont, Tuscany and Marche in 2013 and only in Lombardy in 2014. The "Villupuram" variant represents the only D8 variant ever identified during all the triennium 2013–2015 (in Veneto, Emilia-Romagna, Abruzzo, Basilicata and Apulia in 2013, in

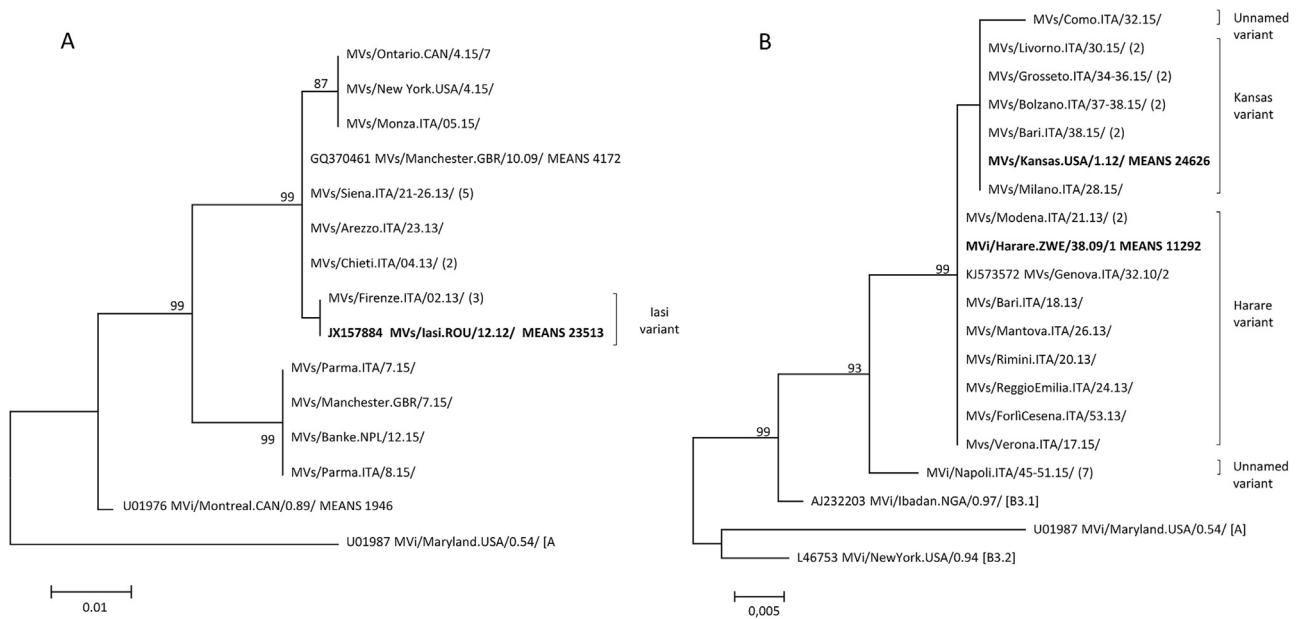


Fig. 3. Neighbour-joining tree for nucleotide sequences of MV strains belonging to genotype D4 (A) and genotype B3 (B). Number in brackets indicate the total number of identical sequences identified in the same province by the same week.

Veneto in 2014 and in Trentino-Alto Adige in 2015). Three more WHO measles variants circulated in 2014: Hulu Langat and an unnamed variant were identified in Lombardy and Rostov on Don variant in Friuli-Venezia Giulia (Fig. 4C). Moreover, the latter was the most representative variant in 2015, being identified in Trentino-Alto Adige, Friuli-Venezia Giulia, Umbria and Sardinia, and involved in an outbreak occurred at an international dog show in Slovenia (MV/Nova Gorica.SVN/47.14/) in November 2014 (Kalaycioglu et al., 2016;

Grgič-Vitek et al., 2015). The WHO named variants described above were reported in the reviewed period in other European countries (Filia et al., 2015; Santibanez et al., 2015; Rasmussen et al., 2015).

3.3. B3

B3 circulated throughout the period 2013–2015: it was identified from 5 cases in 2013, while the genotype co-circulated with genotype

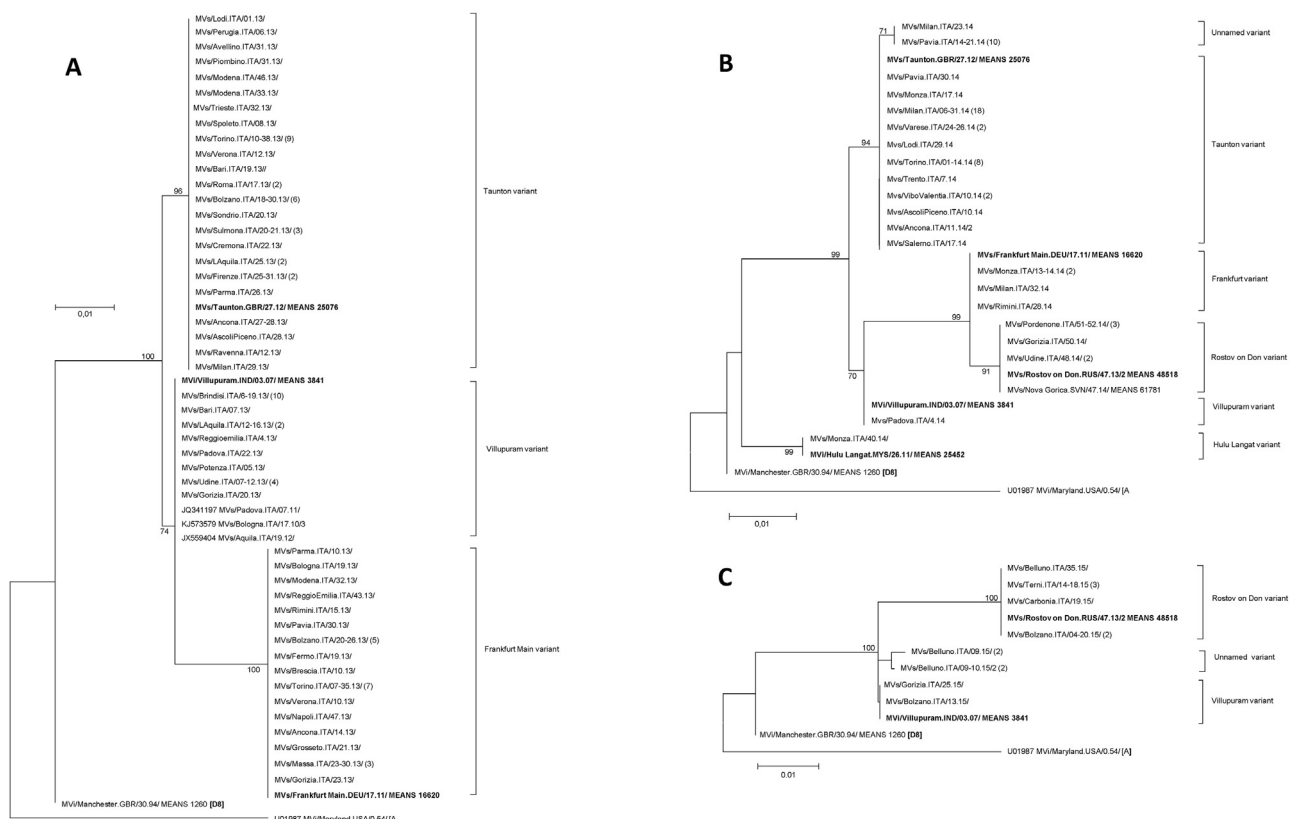


Fig. 4. Neighbour-joining trees for nucleotide sequences of MV strains belonging to genotype D8. A–C refer to the years 2013, 2014 and 2015, respectively. Numbers in brackets indicate the total number of identical sequences identified in the same province by the same week.

D8 and was identified in 164 cases in 2014. In 2015, genotype B3 was responsible of 38 out of 57 (66.7%) characterized cases.

The 8 sequences identified in 2013 (Fig. 3B) were similar to each other and to those already circulating in Northern Italy in 2010 (MV/Genoa.ITA/32.10/2, GenBank KJ573572). These strains all belong to the variant “Harare” (MVi/Harare.ZWE/38.09/).

During 2014, B3 was the most frequently identified genotype, with a percentage of 63.1% (164/260). B3 strains belonged to 3 main WHO named variants (Tonbridge, Harare, Dakar) and to a WHO unnamed variant (proposed as “Ústí nad Labem”), including that responsible for a cruise ship outbreak occurred at the beginning of the year (Lanini et al., 2014). The circulation of these WHO variants has been illustrated (Filia et al., 2015; Magurano et al., 2016; Nic Lochlainn et al., 2016). In 2015, genotype B3 continued to be the most frequent genotype with the variants: “Harare” (MVi/Harare.ZWE/38.09/) in the Veneto, Friuli Venezia Giulia, Apulia, Lombardy and Emilia Romagna regions; “Kansas” (MV/Kansas.USA/1.12/) in Lombardy, Liguria, Trentino Alto Adige, Tuscany, and Apulia. Two more unnamed variants were identified: one in Northern Italy (MV/Como.ITA/32.15/) was responsible for an outbreak occurred in the Roma/Sinti population with transmission in the nosocomial setting, described by Filia et al. (2016); the second was identified in Southern Italy (MV/Napoli.ITA/45.15/).

3.4. D9

Four sequences belonging to genotype D9 were identified in 2013 and 1 in 2014 (Fig. 5). Two out of four sequences of 2013 (MV/Monza.ITA/45.13/) were 100% identical to each other and to the strains identified in Australia (GenBank KF802423) in the same period, belonged to the WHO named variant “Yamanashi” (MV/Yamanashi.JPN/51.12/). Anyway, no epidemiological data are available to determine possible routes of importation for these strains in Italy. One sequence identified in 2013 (MV/Varese.ITA/33.13/) showed 99% of identity with strains circulating in USA (GenBank JX308263), Turkey (Kalaycioglu et al., 2013) and Malaysia (GenBank JQ978702) during 2012. Epidemiological data confirmed that this strain was imported from Australia, where the patient had traveled.

A fourth strain identified in 2013 (MV/Bologna.ITA/41.13/) was 99% similar to that identified in Australia (GenBank KF802423) in the same period, and epidemiological data confirmed the importation of this strain from Indonesia.

The unique D9 sequence identified in 2014 (MV/Bolzano.ITA/43.14/) was identical to the strain MVs/Feldkirch.AUT/42.14/(Means 67972) identified in the same year in Austria. Epidemiological data are not enough to establish a route of importation from Austria.

3.5. H1

Genotype H1 was identified in four measles cases during an

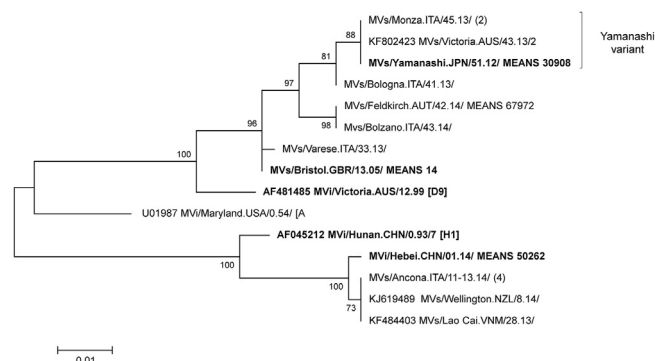


Fig. 5. Neighbour-joining tree for nucleotide sequences of MV strains belonging to genotypes D9 and H1. Numbers in brackets indicate the total number of identical sequences identified in the same province by the same week.

outbreak reported in 2014 in Central Italy (Ancona, Marche) (Fig. 5). The H1 sequences were identical to a strain identified in New Zealand in the same year (MV/Wellington.NZL/8.14/, GenBank KJ619489). Epidemiological data available for these cases was insufficient to establish the route of importation of infection.

4. Discussion and conclusions

Phylogenetic analysis of wild-type MV showed that the genotypes D4 and D8 were endemic in Italy in 2013. Genotype D8 circulated all over the triennium, whereas D4 circulated up to the first half of 2013 and sporadic cases were detected in 2015.

Sporadic MV strains belonging to genotype B3 were detected during 2013, afterwards the genotype became endemic in the next biennium 2014–2015. Sporadic cases of D9 and H1 were identified in 2013 and 2014, probably due to importation from Asian countries where these genotypes are endemic, but there were not epidemiological data to support this hypothesis for all the cases reported. Likewise, one B3 strain (MV/Modena.ITA/21.13/, Harare variant) was probably imported from Africa and introduced in Italy in 2013. The circulation of genotypes in the European WHO Region (data from MeaNS) followed a similar trend with prevalence of D8 and B3 (Fig. 2B).

Virological surveillance represents a fundamental tool to document the interruption of the transmission of endemic measles and needs to be further strengthened in Italy. Timely and accurate laboratory diagnosis, including molecular characterization of circulating measles strains, is essential to identify imported viruses, define import-related outbreaks and understand transmission patterns. Currently, local epidemiological investigation on the outbreak is inadequate. As a consequence, even though the work of NRL/SRLs laboratories allowed to identify MV genotypes responsible for several Italian outbreaks in the triennium, the rate of genomic characterization above the total number of outbreaks reported (which is one of the indicators to evaluate the performance of the national laboratory national system) is far from 80% requested by WHO (10% in 2013, 43.1% in 2014 and 40% in 2015). All these data show that Italy is far from measles elimination.

In order to increase performance level requested by WHO, including timeliness and completeness of data, in March 2017 a sub-national network of accredited labs for measles and rubella (MoRoNet) coordinated by the NRL was formalized (Magurano et al., 2017).

Moreover, Italy is far from the vaccination coverage of at least 95% with two doses of measles vaccine needed to interrupt the circulation of MV and strategies should be reinforced to address the susceptibility to measles of unvaccinated young adults and other population groups.

In conclusion, as recommended by WHO Italy needs to renew political commitment and improve its National Surveillance System to reach the goal of measles elimination (Adamo et al., 2017). Main challenges include low population immunity, consistently low and ever decrease routine vaccination coverage, a complex surveillance system with suboptimal sensitivity and insufficient engagement of proficient laboratory in the surveillance system, in addition to the WHO-accredited laboratory.

This paper describes measles cases reported to the Italian national measles surveillance system between 1st January 2013 and 31st December 2015, and discusses some of the priorities for reaching measles elimination. Since measles elimination in the WHO European Region is a public health priority, it is crucial to share information with all the actors involved in the European public health and underline the severity of measles even in industrialized countries. Further effort and more investments will be needed to allow Italy to become one of the countries that have interrupted endemic measles transmission.

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